1979 Annual Report on Almond Pollination Research

Sponsored by the Almond Board of California.

Title: Tree Research: Pollination (Project No. 79-04)

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Objectives: To develop information on pollination by bees which will result in increased production and greater grower returns.

Interpretive summary: The application of supplemental pollen to be redistributed by adequate numbers of bees was tested for the second year in an orchard with solid blocks of four rows of Nonpareil bordered by NePlus and Mission. This test showed no increase in yield due to artificial pollination.

Methods for early determination of effective pollination are critical to evaluations of all pollination studies. Our preliminary search suggests visible post-pollination changes in color of stigmas and pistil apices, and of petal bases may be useful, although somewhat subjective. Electrophotography of a small sample of pistils showed consistent differences between non-pollinated and open pollinated stigmas and merits further examination. Pollen or nectar foraging honey bees tend to apportion their activities on each variety in relation to the pheological stages (age classes) of

its bloom. Pollen foragers abound when early stages of bloom are abundant. When older blooms are most abundant nectar foragers dominate. This provides some explanation for the higher set of the earliest flowers of each variety since the principal visitors to these should be pollen foragers which appear to be the most efficient in pollen transfer.

Bouquets can improve pollination, especially in orchards with poor planting schemes. Reciprocal bouquet tests suggest bees respond more to phenological differences than to varietal differences in almond flowers. This stresses the importance of coincident bloom among varieties.

Flourescent nectar trials using experimentally modified flowers *(Fremontodendron)* support our hypothesis that bees can perceive and use visual characteristics of nectar to determine its presence.

Pollen transfer efficiency tests tend to support our hypothesis that pollen foragers are more efficient than nectar collectors in almonds.

Studies on the effects of Benomyl on honey bee brood have been initiated and studies of comparative ultrastructural morphology of pollen grains of almond varieties are continuing, but no new information is available on these studies at this time.

Pollination experiments and observations:

Artificial Pollination

Previous controlled test of applications of supplemental pollen to almond orchards with adequate bees to move it around, have not demonstrated increased yields in orchards with recommended plantings of cross-compatible varieties (e.g. 1:2:1 NePlus: Nonpareil: Mission). We conducted an experiment in an orchard with an unfavorable varietal combination planting

(1:4:1 with 4 contiguous rows of Nonpareil) in 1978 that suggested the addition of compatible pollen improved yields. Treated and non-treated plots were reversed in this orchard in 1979 in an attempt to validate this result.

Experimental procedure: The 60 acre test orchard had four rows of Nonpareil bordered by a row of NePlus on one side and Mission on the other. Pollen from Merced and Jordanolo with *Lycopodium* spores (1:3) as the carrier was applied by ground blower with a special auger to meter the material uniformly. Nonpareil rows in the south half of the orchard were treated three times (Table 1) at the rate of 50 grams per acre, those in the north half of the orchard were exposed to air from the blower on the same dates, as a control. Bees at the rate of 2 3/4 hives per acre were distributed throughout the orchard. Bouquets were placed in trees to increase pollination, but these were distributed equally throughout the orchard so as not to interfere with the pollen application test. Bud and flower counts were made on a tagged limb in each of 10 trees in both areas in the pretreatment bloom count, 6 March. On each treatment day an additional 10 limbs each on a separate tree were tagged, receptive flowers were counted and all others were removed. Fruit set counts were made on all tagged limbs in April. Applications were monitored with sticky plates and by collection of flowers and bees. Nonpareil flowers were bagged and hand pollinated with the application material or fresh pollen as an in vivo test of viability.

Results: Fruit set data (Table 2) show that the treated rows had slightly (ca. 1%) lower set than the nontreated rows. Hand pollinations gave only 4.2% set (n=48) with the commercial pollen as compared with 83.3% set (n=12) with fresh pollen from anthers of a compatible variety from the same orchard. Harvest data based on 16 Nonpareil rows in each plot showed no difference: 40 bins each with the total Nonpareil crop down about 200 lbs. from 1978.

Monitoring procedures, while admittedly small samples, detected few *Lycopodium* spores beyond 6 feet from the blower and none in rows adjacent to the one being treated. Thus the dispersal of the applied pollen appeared quite localized, primarily to the lower limbs of the treated trees.

The pollination season in 1979 started later and progressed more rapidly with better overlap between varieties than in 1978. This test merits repeating next season.

Early Determination of Effective Pollination

The purpose of these observations was to identify post-pollination changes in floral pheonology (development) following effective pollination (including fertilization) by compatible pollen. Visible changes as well as techniques to detect changes in temperature, internal development, electrophotographic response were investigated to find a method of early evaluation of pollination effectiveness that would be more suitable than the laborious analyses of styles for pollen tube growth. It may prove a suitable alternative to fruit set counts also.

Experimental procedure: Almond limbs were cut under water and held for 8 days in a screened breezway. Half the flowers on these limbs were hand pollinated with compatible pollen for comparison with the unpollinated flowers. Temperatures of the flowers were recorded with an infra-red sensitive thermometer. A second set of flowers were covered with bags for a month. Temperatures of the nutlets produced were compared with those of nutlets from unbagged flowers on the same tree. Some of these nutlets were X-rayed to evaluate differences in internal structure. Pistils from bagged and open pollinated flowers were compared electrophotographically.

Results: Stigmas and pistils of hand pollinated flowers darkened and withered faster than those of non-pollinated ones (Table 3). Petal bases of hand pollinated flowers reddened faster than those of nonpollinated ones, but the latter dropped their petals more rapidly in Trial B (Table 3).

No significant differences in temperature were detected between hand pollinated and non-pollinated flowers although all flowers were consistently above ambient air temperatures by 1 to 2°C temperatures. Nutlets from non-pollinated flowers had slightly but consistently higher (by 0.2-0.3°C) temperatures than open pollinated ones.

X-ray radiography of non-pollinated nutlets compared with open pollinated showed little consistant difference, but a higher proportion of non-pollinated nutlets were uniformly amorphous internally. Electrophotography (Kirlian photography) of pistils from non-pollinated flowers consistently showed a glow in the area of the stigma which was not present in pistils of open pollinated flowers. This appears to merit further investigation as a rapid, inexpensive method of obtaining a permanent record for early determination of effective pollination.

Flower Age Preferences of Bees

We have established that foraging behavior of honey bees differs depending on whether they are gathering pollen or nectar. In 1978 we determined that these differences were related to the phenological stages we had identified and described in 1977. We then wanted to determine how the tendency of nectar collectors to visit older flowers than do pollen collectors relates to differences between varieties on the same date and to the phenological changes in bloom of a variety through time.

Experimental procedure: Counts were made of over 100 flowers per variety to determine the percentage in each of 6 phenological (age) categories. Counts of bees were then made by observers walking slowly along the south side of a tree row noting the number of pollen vs. nectar foragers during 5 to 15 minute periods.

Results: At any point in time the percentage of nectar gathers was highest on the earliest blooming varieties which were at or past peak bloom while pollen foragers tended to be relatively more abundant on the later blooming varieties (Table 4). This is correlated with flower age class frequencies. By 13 March most of the foragers observed were nectar gatherers. Since pollen foragers appear to be more efficient pollinators, these observations may indicate why we have found that early opening flowers of a variety have the highest percent fruit set.

Bouquet Pollination

Bouquets of compatible blossoms were placed in an orchard with a poor planting scheme to determine if fruit set could be improved. Fruit set counts were also made in the vicinity of our reciprocal bouquet experiments (see next section) in an orchard with adquate varietal interplanting to determine if pollination could be enhanced even with adequate pollination conditions.

Experimental procedure: In an orchard with the east half of each row planted with Mission and the west half with NePlus, 3 types of bouquets in water with floral preservative were tested: single limbs placed in bottles were hung on tree limbs 1 or 2 to a tree; buckets of several limbs were placed on the ground beneath the canopy of a tree; or bucket bouquets were placed in the crotches of trees. Fruit set counts were made at 6" to 36" from all bouquets and in a non-treated row in a remote corner of the orchard. Fruit set counts were also taken in the canopy in the rows with bucket bouquets, in the intervening untreated 2 rows.

In another orchard with adquate interplanting reciprocal bouquets were set up to test varietal preferences of bees (see next section). Fruit set counts were made in the trees where the bouquets had been placed and an untreated tree of the same variety.

In a third orchard with bucket bouquets on the ground and in the crotches of trees, observations were made on foraging behavior of bees in the vicinity.

Results: In the orchard inadequate varietal interplanting, the percent fruit set was highest nearest the bouquets, but decreased sharply away from them (Table 5). However samples were small and not always consistent. In the reciprocal bouquet tests, fruit set in treated trees averaged about 39-41% while set in untreated trees averaged about 34%, but again there was high variability among measurements.

Observations on foraging behavior showed more frequent visitation to buckets in tree crotches than those on the ground. Bees working bouquets in tree crotches also visited flowers of that tree, but bees foraging on ground bouquets were not seen flying to trees.

Reciprocal Bouquet Experiments

These experiments were designed to test varietal discrimination and preference among bees foraging in almonds.

Experimental procedure: Single cut branch bouquets in bottles with water and floral preservative were placed in trees. Each host tree contained bouquets of two different varieties, one of the same variety and an uncut control branch. All but receptive flowers were removed from each branch to be observed. Bee visitiation was observed and landing-probing vs. approach-avoid behaviors were recorded.

Results: Analyses of the landing-probing behavior on the host tree branch compared with a cut branch of the same variety and branches of two other varieties in the same tree showed no significant differences in the means of among-groups vs. within groups since F was less than unity (Table 6). Analyses of approach and avoid behavior also showed no significant differences among- vs. within-groups (Table 6). These data suggest that bees exhibit little or no discrimination among almond flowers of different varieties as long as they are similar in phenological development (age). This reinforces our earlier findings of the importance of floral phenology in relation to bee preference and foraging behavior. Thus, the need for coincidence of bloom between compatible varieties becomes of utmost importance.

Fluorescent Nectar

Our hypothesis (1975) that the fluorescent or ultraviolet absorption characteristics of nectar of almond flowers (and of some other bee visited flowers) can be perceived and used by foraging bees has been tested with artificial flower models (1976-1978). To further validate this hypothesis a test with experimentally modified flowers was initiated.

Experimental procedures: To minimize interference of these tests with gathering of other data during almond bloom, we selected flowers of *Fremontodendron californica,* flannel bush, as our test sytem since their nectar fluoresces blue like that of almonds and they bloom in April and

May. Nectar was collected early in the morning before the bees became active. Flowers of the same age (phenological stage) were transferred to florists vials containing water and flower preservative. The anthers were removed and the nectaries were flushed with distilled water and dried by absorbing all liquid with tissue paper. The nectaries were refilled with previously extracted nectar, water or nothing. Experimental flowers were attached to the limbs of the host shrub and bee visitation behavior was recorded. Two sets of the three treatments were monitored by each observer. Treatments were rotated among the three flowers of each set every 10-15 minutes to avoid any possible bias due to contamination by previous bee visits and position effects.

Results: The bee visitation based on landings and probings at flowers differed significantly ($P<0.0005$) for each date and for total observations from that expected if they perceived all treated flowers the same (Table 7). This was due to a strong bias toward visits to the flowers with nectar. Chi-square values for all comparisons of landings at flowers filled with nectar added versus those with water added or those without liquid differed significantly ($P<0.0005$) while values for flowers filled with water versus those without liquid did not differ significantly. Approach and avoid behavior observations showed almost no significant differences, except due to nectar added versus empty nectaries on 16 May ($P<0.01$). Thus, the bees showed strong preference to visit flowers with fluorescent nectar present, but did not discriminate between flowers with nonfluorescent liquid and those without liquid in their nectaries.

Pollen Transfer Efficiency

Previous observations demonstrated that bees foraging for pollen on almond flowers contact stigmas more frequently than do nectar foragers and therefore may be more effective pollinators. The following preliminary observations were designed to determine whether pollen collectors had amounts of pollen on their bodies different from those on nectar foragers.

Experimental procedures: Hoeny bees captured while foraging on almond flowers had their ventral sides daubed on double-stick scotch tape on a microscope slide. Others were also pressed against amond stigmas from which other pollinators were excluded.

Results: A greater frequency of pollen foragers tended to have large amounts of pollen on their venters than that of nectar foragers (Table 8). Better fruit sets were obtained by daubing pollen collecting honey bees on stigmas than by using nectar foragers (Table 9), however the samples are extremely small and more such testing seems warranted.

Effects of Benomyl on Honey Bee Brood

A study to determine the effects of the fungicide Benomyl on developing brood of honey bees has been initiated, but no analyses of the data are available at this time.

Pollen Morphology

The comparative study of ultrastructural characterisitcs of pollen grains from different almond varieties using scanning electron microscopy is continuing on a "time available" basis.

Publications

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Table 1. Artificial pollination. Percent of bloom before and during applications of pollen to Nonpareil trees.

	Date	Treated Rows	Nontreated Rows
Pretreatment:	5 March	73.5% (1503)	73.7% (1227)
Treatment 1:	6 March	79.3 (1060)	65.8 (1649)
Treatment 2:	7 March	89.6 (2295)	75.3 (1739)
Treatment 3:	9 March	90.9 (1969)	92.0 (2107)

Table 2. Artificial pollination. Percent fruit set on tagged limbs on each of 10 Nonpareil trees for each date and plot.

	Original Counts	Treated Rows	Nontreated Rows
Pre-T	5 March	6.7% (1503)	11.2% (1227)
T-1	6 March	5.3 (825)	5.0 (1129)
T-2	7 March	2.9 (1237)	6.1 (920)
T-3	9 March	9.9 (961)	10.3 (707)

Table 3. Post-pollination changes. Color changes in the base of petals and in the stigma and apex of pistil in hand pollinated (HP) versus non-pollinated (NP) almond flowers on cut limbs. Flower age groups: Y=youngest; I=intermediate; O=oldest.

	Petal Color						Pistil Color					
Trial A	NP			HP			NP			HP		
March	Y	I	O	Y	I	O	Y	I	O	Y	I	O
13	9	3		2	8	2	12			12		
15	6	6		1	6	5	12			10	2	
16	6	6		1	5	6	12			7	3	2
18	6	5	1	-	3	9	12			4	1	7
19	6	5	1	1	3	8	12			5	-	7
20	2	7	3	-	2	9[a]	11	1		3	-	8[a]
21	3	8	1	-	2	8[b]	8	2	2	4	-	8
Trail B March												
20							10			10		
21							10			8	2	
22	4	2	1[c]	4	-	6	8	2		6	2	2
23	4	2	1[c]	-	5	5	9	1		4	3	3
28	3	3	[d]	-	-	9	4	3	2[e]	-	1	9

[a] 1 flower not recorded

[b] 2 flowers without petals

[c] 3 flowers without petals

[d] 4 flowers without petals

[e] 1 flower missing

Table 4. Flower age preferences of bees. Percentages of nectar vs. pollen collecting bees visiting varieties with various percentages of flowers in different phenological (age) categories. (N=numbers of flowers or bees observed).

	9 March			10 March				
Age	NePlus	Nonpareil	Mission	NePlus	Peerless	Thompson	Drake	Mission
Bud	0	1.8	37	0.9	0	4.9	0.8	12.6
Cup	0	0	1.9	0	0	1	0.8	3.5
<50% Dehisc.	0	1.8	14.8	1.4	3.5	10.8	0.8	19.8
>50% Dehisc.	0.8	16.5	40.7	13.6	37.2	76.5	70.3	62.6
E. Senesc.	5.9	10.1	4.6	55.7	49.6	6.9	6.6	1.6
L. Senesc.	93.7	69.7	0.9	28.5	9.7	0	20.7	0
(n=)	(118)	(109)	(108)	(221)	(113)	(102)	(121)	(374)
Bees: Pollen	0	34.8	95.5	6.3	17.6	41.2	88.9	77.4
Nectar	100	65.2	4.5	93.8	82.4	58.8	11.1	22.6
(n=)	(17)	(23)	(66)	(16)	(17)	(17)	(81)	(358)

	12 March						13 March		
Age	NePlus	Peerless	Drake	Thompson	Merced	Mission	Nonpareil	Thompson	Mission
Bud	0	0	0	0	0	13.2	0	0.4	5.6
Cup	0	0	0	0	0	2.8	0	0	5.6
<50% Dehisc.	0.9	1.8	0	4.6	0.8	13.9	1.4	4.8	26.1
>50% Dehisc.	4.3	21.4	40.6	28.7	26.4	37.5	3.8	8.1	40.3
E. Senesc.	51.7	72.3	59.4	66.7	60.8	32.6	25.8	38.5	19.5
L. Senesc.	43.1	4.7	0	0	12	0	69	48.4	3
(n=)	(116)	(112)	(106)	(108)	(125)	(144)	(287)	(273)	(303)
Bees: Pollen	5	16.7	22	41	59.4	61.6	11.1	4.6	6.5
Nectar	95	83.3	78	59	40.6	38.4	88.9	95.4	93.5
(n=)	(20)	(24)	(41)	(39)	(64)	(73)	(45)	(108)	(138)

Table 5. Bouquet pollination. Percent fruit set at different distances from bouquet treatments.

Bucket bouquets		Untreated Trees: Test tree	Untreated Trees: Near	Untreated Trees: Far	Bottle bouquets	Test Tree
in crotch	Crotch	12.3%	6.4%	-	Distance 6-9 (inches)	23.9%
	Canopy	7.3	5.8%	-	12-18	11.6
on ground	Crotch	6.5	-	-	24-36	9.5
	Canopy	7.1	-	-	Mean	13.8
	Mean	8.2%	6.1%	2.0%		

Table 6. Reciprocal bouquet test. Data summary and F-test for differences of mean squares among-groups vs. within-troups for land & probe, and approach & avoid data.

		Host Limb	Cut limbs: Same var.	Cut limbs: Other vars.	
Land & Probe	n	16	16	32	F=0.101
	ΣX	464	409	874	
	$\bar{x}$	29	25.6	27.3	
	s^2	550.47	277.33	521.64	
	s	23.46	16.65	22.84	
	ΣX^2	21,698	14,615	40,042	
Approach & Avoid	n	16	16	32	F=0.315
	ΣX	223	260	557	
	$\bar{x}$	13.9	16.3	17.4	
	s^2	173.93	188.07	226.19	
	s	13.19	13.37	15.04	
	ΣX^2	5,717	7,046	16,707	

Table 7. Fluorescent nectar. Honey bee visitation behavior to experimentally modified *Fremontodendron* flower nectaries: N=nectar added; W=water added; E=empty (without liquid) at Davis, CA

Land & Probe

Date	N	W	E	Totals	Expected	χ^2 (df=2)	P<
May 16	51	7	4	62	20.7	66.9	0.0005
17	68	26	23	117	39	32.5	0.0005
18	52	20	15	87	29	27.8	0.0005
Totals	171	53	42	266	88.7	115.3	0.0005

Approach & Avoid

Date	N	W	E	Totals	Expected	χ^2 (df=2)	P<
May 16	20	32	41	93	31	7.1	0.05
17	59	72	65	196	65.3	1.4	0.50
18	28	30	43	101	33.7	3.9	0.30
Totals	107	134	149	390	130	7.0	0.05

Table 8. Pollen transfer efficiency. Frequencies of honey bees with different amounts of pollen on venter. Bees collecting pollen (P); nectar (N); and both (P&N).

No Grains	P	N	P&N
10-100	1	4	1
100-1,000	21	13	3
1,000-10,000	8	1	0

Table 9. Pollen transfer efficiency. Fruit set percentages from daubing honey bees collected while foraging for pollen (P) or nectar (N).

Variety	P	(n)	N	(n)
Other	22.2%	(18)	18.8%	(16)
Same	16.7	(6)	0	(8)
Total	20.8	(24)	12.5	(24)